

Purification of Triglycerides With an Alumina Column¹

SYNTHETIC MIXED ACID triglycerides (TG's) are usually prepared by acylating mono- (MG's) or diglycerides (DG's) with the requisite fatty acid chloride. If acylation is incomplete, a mixture of varying quantities of tri-, di- and monoglycerides and fatty acid results. In our hands, the TG content of acylation mixtures has ranged from about 55-90%. Purification of these acylation mixtures can be difficult and laborious as silicic acid column chromatography will handle relatively small quantities and crystallization is often wasteful with accompanying reduction in yield. Chapman et al. (1) avoided these difficulties to a large extent by using a column of neutral alumina to remove the impurities (DG's) from their preparations of synthetic TG's.

We purified preparations of glyceryl-1-palmitate-2,3-dibutyrate (PBB) with the column as this compound cannot be crystallized except at very low temperatures (-96C). We have since purified many synthetic and natural TG's with the alumina column. The data accumulated indicates that the technique will clean up relatively large quantities, with good recovery and without structural alterations. We believe the procedure will be useful to others, hence this report.

Neutral alumina, Brockman activity I (Fisher) Alcoa alumina (F-20 for chromatography) and heated Alcoa alumina (260C, 12 hr) all performed equally. We use the Alcoa alumina because it is relatively inexpensive. The alumina is routinely heated at 260C for 12 hr in batches after removal from the drum. It is then kept in a tightly sealed jar. Any standard chromatographic tube will suffice to hold the alumina which is usually added at the rate of 2 g per gram of material to be purified. The dry alumina is wetted with petroleum ether (30-60C) or a mixture of petroleum ether-ethyl (9:1), the lipid material dissolved in a minimum amount of ether solvent and placed on the column. The TG's are eluted with 200 ml of solvent per 10 g of material and 20 g of alumina. Methyl esters or other compounds less polar than TG's are also eluted, while diglycerides, free fatty acids, etc., remain on the column. The whole process requires about 15 min and eluted TG's have always been colorless. The process can be scaled up. We added 81.6 g of alumina and 106.8 g of impure synthetic glyceryl 1-palmitate-2-oleate-3-

stearate (POS) to a column 3 cm in diameter and recovered 100.4 g of TG, pure by thin-layer chromatography (TLC), with 400 ml of petroleum ether-ethyl ether 9:1. The POS had been crystallized to 95% purity as estimated by TLC and contained DG's and fatty acid. Ninety nine grams of TG was recovered in the first 200 ml of solvent, 1.4 g in the second 200 ml.

Diglycerides can be eluted with 1:1 petroleum ether-ethyl ether, but they are recovered as a mixture of isomers. We have used this to produce mixtures of 1,2- and 1,3-isomers from pure 1,3-diglycerides.

Recoveries of purified olive oil and triolein (Hormel) all TG by TLC, ranged from 85-90%. Other recoveries based on the original weight of the impure mixture with quantity (%) of TG in the mixture given in parentheses were: tributyrin, 75 (80); glyceryl-1-oleate-2,3-dicaproate, 63.3 (75); glyceryl-1-oleate-2,3-dibutyrate, 66.0 (75); glyceryl-1-oleate-2,3-dipalmitate, 56.0 (70); glyceryl-2-oleate-1,3-dipalmitate, 80.0 (93); glyceryl-1-palmitate-2,3-dioleate, 75.3 (89); glyceryl-2-palmitate 1,3-dioleate, 75.0 (85.0); SOP, 94.0 (95); glyceryl-1-oleate-2,3-dimyristate, 88.7 (96); and glyceryl-1-stearate-2,3-dioleate, 92.5 (95). In general, the TG's were synthesized as described by Mattson and Volpenhein (2). Purity before and after column treatment was ascertained with TLC. Use of the column to purify acylation mixtures eliminates washing with dilute base to remove fatty acids and consequently eliminates the troublesome emulsions that often

TABLE I
Fatty Acid Composition of Triglycerides Purified with an Alumina Column and of the Free Fatty Acids and Monoglycerides Derived Therefrom by Pancreatic Lipolysis

Triglyceride ^a	Fraction	Fatty acid (M%)		
		16:0	18:0	18:1
SOO	Intact TG ^b	33.7	66.3
	MG	0.3	99.7
POP	TG	66.6	33.4
	FFA ^b	99.1	0.9
OPP	TG	66.7	33.3
	FFA	50.0	50.0
POO	MG	98.8	1.2
	TG	33.3	66.7
OPO	FFA	49.8	50.2
	MG	>98.5
SOP	TG	33.0	67.0
	FFA	>99.0
	MG	99.0
	TG	33.8	33.1	33.1
	FFA	50.3	49.7
	MG	>99.0

^a S- stearate, O- oleate, P- palmitate.

^b TG- triglyceride, MG- monoglyceride, FFA- free fatty acids.

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TABLE II
Effect of Purification with an Alumina Column on Fatty
Acid Position in Olive Oil

Fatty acid	MG ^a		FFA ^b	
	Pre	Post	Pre	Post
	M%			
16:0	0.6	0.5	19.8	20.4
16:1	0.6	0.5	0.5	1.1
18:0	0.4	0.1	4.1	4.6
18:1	85.5	88.3	69.4	69.4
18:2	11.9	9.9	5.2	3.6
19:0	0.4	0.5
18:3	1.0	0.7	0.6	0.3

^a Olive oil eluted from column of alumina with 9:1 petroleum ether-ethyl ether.

^b MG- monoglyceride, FFA- free fatty acids.

arise. If the TG content is less than about 50% then absolute recoveries are depressed.

Exposure of double bonds to alumina has been reported to result in positional isomerism (2). To test this, triolein (Hormel) was passed through the alumina column, converted to methyl esters, examined for *trans* isomers by infrared spectrophotometry and for positional isomers by oxidation as described by Tinoco and Miljanich (3). *Trans* isomers were not detected. Further there was no difference between the oxidation patterns as determined by gas-liquid chromatography (GLC) of the original and that after passage through alumina. The major peaks obtained corresponded to retention times of authentic methyl azelate, oleate, and pelargonate. Dicarboxylic acids other than ~~pelargonate~~ were not detected. We concluded that the alumina column did not cause isomerization. This experiment was repeated on methyl linoleate (Hormel) with similar results and the same conclusion.

To determine if alumina affected positional integrity we examined some of the synthetic TGs above with pancreatic lipolysis. In addition, purified olive oil was similarly checked before and after column treatment. The fatty acid composition (GLC) of the resulting free

fatty acids and monoglycerides from the synthetic TG's in Table I and from the olive oil in Table II indicates that the alumina column did not cause disproportionation of the fatty acids. Many other synthetic TG's containing a large variety of acids; short-chain, *trans* isomers, etc., have been tested with pancreatic lipolysis after purification and positional purity was maintained.

The virtues of the column are apparent. Purification of TG's from acylation mixtures, particularly those that are difficult to crystallize, is rapidly achieved without alterations in structure. Recoveries of TG's from these mixtures are close to 90%. Further, no difficulties are caused if the column runs dry. If however, the mixture to be purified is not readily soluble in the eluting solvent, flow is delayed and purification does not occur. Nevertheless, the advantages of speed, good recovery, lack of structural alterations and large column load should recommend its widespread application.

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